

Amendments to the Claims

This listing of claims replaces all prior versions and listings of claims in the application. Any amendments or cancellations are made without prejudice or disclaimer.

Listing of Claims:

1. (Cancelled)
2. (Currently amended) An isolated protein that has ~~The~~ the glycerol kinase activity, the protein comprising the following physical and chemical properties:
 - (1) the activity of the protein has the ability to modify glycerol, in the presence of ATP, to glycerol-3-phosphoric acid;
 - (2) the activity of the protein is optimal at a pH of about 10.0;
 - (3) the activity of the protein is optimal at a temperature of about 50°C in a reaction carried out for five minutes in the presence of 20 mM HEPES buffer at a pH of about 7.9;
 - (4) at least 90% or more of the activity of the protein is retained after incubation of the protein at 25°C for two hours at a pH of about 6.0 to about 10.0;
 - (5) at least 90% or more of the activity of the protein is retained after incubation of the protein for about 15 minutes in 50 mM potassium phosphate buffer at a pH of about 7.5;
 - (6) the protein contains a subunit that has a molecular weight of about 55,000 daltons as determined by sodium-dodecyl sulfate polyacrylamide gel electrophoresis or the intact protein has a molecular weight of about 176,000 daltons as determined by gel filtration;

(7) the glycerol kinase activity has a K_m of about 6.9×10^{-6} M for glycerol and a K_m of about 1.11×10^{-4} M for ATP;

(8) the protein has a relative activity of about 41.2 Units/mg; and

(9) at least 70% or more of the kinase activity of the protein is retained when the protein is incubated for one week at 25°C in the presence of 100 mg/L of N-methylisothiazolone or derivatives thereof.

~~according to claim 1, wherein the resistance against preservative expressed as a remaining activity ratio is 70% or more when the glycerol kinase coexists with the preservative at 25°C for one week.~~

3. (Cancelled)

4. (Currently amended) The isolated protein ~~The glycerol kinase~~ according to claim [[1]] 2, which is a protein of (a) or (b) below:

(a) a protein consisting of an amino acid sequence represented by SEQ ID NO:1 in the Sequence Listing; or

(b) a protein comprising ~~an amino acid sequence of~~ the amino acid sequence of (a), wherein ~~in which~~ one or several amino acids are deleted, substituted or added and having glycerol kinase activity.

5. (Withdrawn-currently amended) ~~A gene encoding a glycerol kinase which is a~~ protein consisting of an amino acid represented by SEQ ID NO:1 in the Sequence Listing.

6. (Withdrawn-currently amended) A gene ~~encoding glycerol kinase~~ consisting of DNA of (c) or (d) below:

(c) a DNA consisting of a nucleotide sequence represented by SEQ ID NO:2 in the Sequence Listing; or

(d) a DNA comprising a nucleotide sequence of the nucleotide sequence (c) wherein one or several nucleotides are added, deleted or substituted and wherein the DNA encodes ~~encoding~~ a protein having glycerol kinase activity.

7. (Withdrawn-currently amended) A recombinant vector comprising a gene encoding the isolated protein glycerol kinase according to any one of claims 1-~~or~~2 or 4.

8. (Withdrawn) A transformant comprising a host cell transformed with the recombinant vector according to claim 7.

9. (Withdrawn-currently amended) A method for preparing an isolated protein a glycerol kinase, which comprises culturing the transformant according to claim 8 to produce an isolated protein a glycerol kinase, and collecting the isolated protein glycerol kinase.

10. (Withdrawn-currently amended) A recombinant vector comprising a gene encoding the glycerol kinase according to claim ~~[[3]]~~ 4.

11. (Withdrawn-previously presented) A transformant comprising a host cell transformed with the recombinant vector according to claim 10.

12. (Withdrawn-previously presented) A method for preparing an isolated protein a glycerol kinase, which comprises culturing the transformant according to claim 11 to produce an isolated protein a glycerol kinase, and collecting the isolated protein glycerol kinase.